

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6:

C12N 15/12, C07K 14/705, 16/28

A2

(11) International Publication Number:

WO 96/09386

(43) International Publication Date:

28 March 1996 (28.03.96)

(21) International Application Number:

PCT/US95/12002

(22) International Filing Date:

21 September 1995 (21.09.95)

(30) Priority Data:

08/310,271

21 September 1994 (21.09.94) US

(71) Applicant: THE GOVERNMENT OF THE UNITED STATES OF AMERICA, re [US/US]; presented by THE SECRETARY, DEPARTMENT OF HEALTH, AND HUMAN SERVICES, National Institutes of Health Office of Technology Transfer 6011 Executive Boulevard, Ste 325, Rockville, MD 20852 (US).

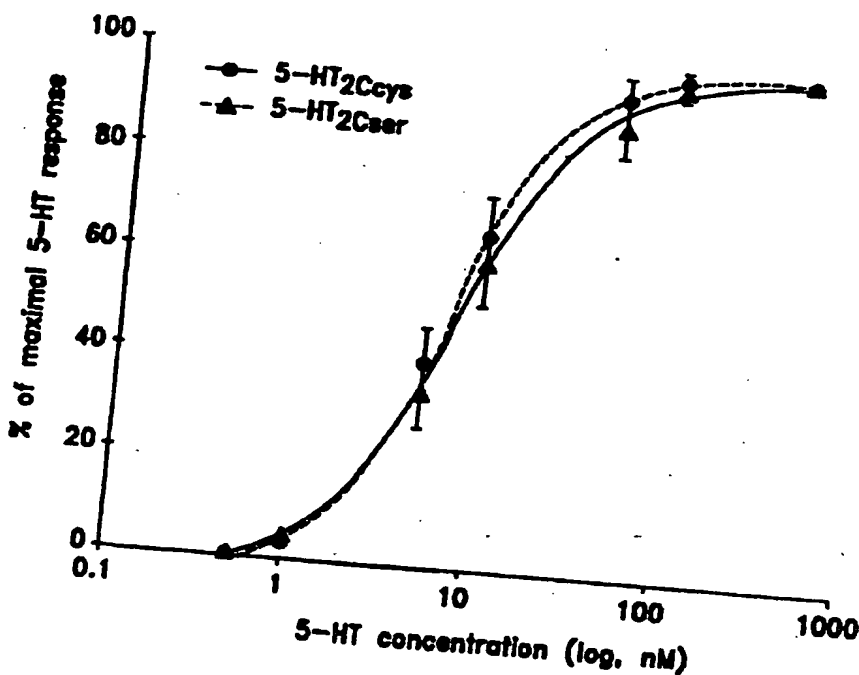
(72) Inventors: LAPPALAINEN, Jaakko; 8200 Wisconsin Avenue, A 1717, Bethesda, MD 20814 (US). LINNOILA, Markku; 7912 Charleston Court, Bethesda, MD 20817 (US). GOLDMAN, David; 12950 Glen Mill Road, Potomac, MD 20859 (US).

(74) Agent: ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, Suite 1600, 620 Newport Center Drive, Newport Beach, CA 92660 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: ALLELIC VARIATION OF THE SEROTONIN 5HT_{2C} RECEPTOR

(57) Abstract

Isolated DNA encoding the serotonin 5HT_{2C} receptor with serine at amino acid position 23, antibody specific to such a variant receptor and the variant receptor protein itself are used in methods for detecting the presence of an allelic variant of the serotonin 5HT_{2C} gene or receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

ALLELIC VARIATION OF THE SEROTONIN 5HT_{2C} RECEPTORField of the Invention

This invention relates to serotonin receptors. Specifically, this invention relates to allelic variants of the serotonin 5HT_{2C} receptor.

5

Background of the Invention

Family, twin and adoption studies indicate genetic vulnerability to psychiatric disorders, including antisocial personality (Crowe, *Arch. Gen. Psychiatry* 31:785, 1974), suicidal behavior (Roy et al., *Arch. Gen. Psychiatry* 48:29, 1991), panic disorder and anxiety. Family and population studies have shown that these disorders usually co-occur with alcoholism and alcohol abuse (Roy et al., *Prog. Neuro-Psychopharmacol. Psychiatry* 11:173, 1987). Although psychiatric disorders often arise from a complex combination of environmental, genetic and biological factors, it may be possible to find biochemical and genetic variables that predict these behaviors and also facilitate implementation of preventative and therapeutic measures.

Several lines of evidence suggest that a lower activity of brain serotonergic pathways is related to several neuropsychiatric disorders. For example, lower levels of 5-hydroxyindoleacetic (5-HIAA), the main metabolite of serotonin (5-HT) in the cerebrospinal fluid (CSF) has been reported in clinical studies of aggression, depression, impulsive crime and alcoholism. In the genetic hypothesis of these disorders, vulnerability is transmitted through inheritance of a functionally divergent allele. Variation in central serotonin function would have pleiotropic effects beyond behavior. For example, some effects would be increasing the vulnerability to anxiety and mood disorders as well as impulsive/aggressive behaviors.

15

Several important genes for normal brain serotonin function have been cloned, including tryptophan hydroxylase (Stoll and Goldman, *J. Neurosci. Res.* 28:457, 1991), serotonin transporter (Hoffman et al., *Science* 254:578, 1991) monoamine oxidases A and B (Bach et al., *Proc. Natl. Acad. Sci. USA* 85:4934, 1988) and several serotonin receptors (Humphrey et al., *Trends Pharmacol. Sci.* 14:233, 1993).

20

Serotonin acts *in vivo* by binding to specific receptors located in the central nervous system. A vast array of serotonin receptors has already been discovered. Investigators have divided the serotonin receptor subtypes into four pharmacologically distinct classes designated 5-HT₁ to 5-HT₄. The 5-HT₁ subcategory contains five different subtypes referred to as 5HT_{1A-E}. The 5-HT₂ receptors can be divided into three subclasses, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}. The primary structures for a number of these receptors have been elucidated by molecular cloning, including the 5-HT₁, 5-HT₂ and 5HT₃ subclasses.

25

Serotonin receptor agonist and antagonists have been developed as drugs for treating specific neuropsychiatric disorders. Drugs with affinity for 5-HT₂ receptors are used to treat schizophrenia, Parkinsonism, and anxiety disorders. Several azapirones, such as buspirone, gepirone, and ipsapirone, have high affinities for 5HT_{1A} receptors in the brain, and are used to treat anxiety. For example, clozapine which is an antipsychotic drug with fewer extrapyramidal side effects is used to treat schizophrenic patients who do not respond to other drug

30

35

treatments. Clozapine has a strong affinity for the serotonin 5-HT₂ subclass of receptors. In addition, 5-HT_{1A} class agonists, such as buspirone, are effective treatments for anxiety.

Highly selective 5-HT uptake inhibitors, which have minimal effects on norepinephrine or dopamine uptake or on other neurotransmitter receptors, have been used successfully to treat depression. Naturally, characterizing all of the specific 5-HT receptors would clarify the role of serotonin in the central nervous system, and assist neuropsychiatric drug development.

The 5-HT_{2C} serotonin receptor subtype has been particularly interesting to investigators searching for the molecular bases of neuropsychiatric disorders. The 5-HT_{2C} receptor gene is widely expressed in the brain where it is involved in regulating endocrine responses. Particular responses include the production and secretion of adrenocorticotrophic hormone (ACTH), oxytocin and prolactin. Genes for rat, mouse and human (Saltzman et al., *Biochem. Biophys. Res. Commun.* 181:1469, 1991) 5-HT_{2C} receptors have been cloned. The functional state of 5-HT_{2C} receptors in normal controls and various patient groups has been studied *in vivo* by administering mCPP, a non-selective 5-HT_{2C} agonist, and measuring hormonal and psychological responses. In alcoholism, panic disorder, seasonal affective disorder and obsessive-compulsive disorder, mCPP has been shown to induce different hormonal and psychological responses in patients and controls.

As discussed, several lines of evidence suggest abnormal function of serotonin receptors in certain neuropsychiatric disorders. Specifically, pharmacological studies in humans suggest that abnormal function of 5-HT_{2C} receptors play a role in the etiology of certain disorders. Accordingly, there is a need to identify and characterize the serotonin receptors and those functional variants which associate with neuropsychiatric disorders. There is a corresponding need for assays that will permit identification of functional variants in various segments of the population. With this knowledge, receptor variant-specific drugs and diagnostic information can be developed.

Brief Description of the Figures

Figures 1A and 1B are a set of hydrophobicity plots. Figure 1A corresponds to the human 5-HT_{2C}cys receptor. Figure 1B corresponds to the human 5-HT_{2C}ser receptors. The X-axis represents the amino acid number of the human 5-HT_{2C} receptor.

Figure 2 is a diagram showing the genetic location of human 5-HT_{2C} receptor gene (HTR2C). Multipoint analysis placed the 5-HT_{2C} gene on the long arm of the X chromosome and in the interval between DXS362 and DXS42 with odds greater than 1000:1. Sex averaged intervals between the HTR2C gene locus and markers are shown.

Figures 3A and 3B are plots showing the relative current potentials activated by 10 nM 5-HT in oocytes expressing either 5-HT_{2C}ser (Figure 3B) or 5-HT_{2C}cys (Figure 3A). 5-HT activated currents were suppressed in the presence of 1 μ M 1-(1-naphthyl) piperazine HCL (PPRZ). The effect of PPRZ was reversible after a 20 min wash.

Figure 4 is a dose-response curve for 5-HT activated currents in oocytes expressing either 5-HT_{2C}cys (●) or 5-HT_{2C}ser (▲).

Summary of the Invention

One embodiment of the present invention relates to isolated DNA encoding that serotonin 5HT_{2C} receptor wherein the DNA encodes a serine at amino acid position 23 of the receptor. The isolated DNA may, for example, be provided in a recombinant vector. Preferably the isolated DNA has the nucleic acid sequence of SEQ ID NO:1.

Also contemplated in the present invention is an isolated protein having the amino acid sequence of a serotonin 5HT_{2C} receptor, wherein the protein has a serine residue at amino acid position 23. Preferably, the protein has the amino acid sequence of SEQ ID NO:2.

Assays for the variant serotonin receptor have significant value, both in research relating to serotonin function and in diagnostic assays because of the correlation between behavioral disorders and this receptor. Thus, the invention includes a method for detecting the presence of DNA encoding an allelic variant of the serotonin 5HT_{2C} receptor, comprising isolating DNA encoding the serotonin 5HT_{2C} receptor, amplifying a region of the DNA that encodes amino acid number 23 of the serotonin 5HT_{2C} receptor, and determining whether the isolated DNA encodes a serine residue at amino acid number 23, wherein a serine residue at amino acid position 23 indicates the presence of the allelic variant of the serotonin 5HT_{2C} receptor. In one embodiment, the amplifying step comprises polymerase chain reaction amplification. This amplifying step may advantageously use DNA primers that insert a new restriction site near the codon that encodes amino acid number 23. For example, in one embodiment, the restriction site is cleaved by *Hinf*I. In a particular exemplification, the DNA primers are SEQ ID NOs 5 and 6. The determining step of the assay may advantageously be restriction enzyme digestion followed by gel electrophoresis, and may optionally include denaturation of the isolated DNA. One suitable restriction enzyme is *Rsa* I. A preferred gel electrophoresis utilizes a Mutation Detection Enhancement gel. In another embodiment, the determining step further comprises nucleic acid sequencing of the amplified DNA.

The present invention further includes antibody specific for the serotonin 5HT_{2C} allelic variant receptor protein wherein the receptor protein has a serine at amino acid position 23. This antibody does not bind the predominant wild-type receptor. Advantageously, the antibody is a monoclonal antibody.

These antibodies, in turn, provide a method for detecting the presence of an allelic variant of the serotonin 5HT_{2C} receptor, comprising providing a biological sample containing the serotonin 5HT_{2C} receptor, contacting the sample with an antibody which specifically binds to a serotonin 5HT_{2C} receptor with a serine residue located at amino acid position 23, and detecting the binding of the antibody to the receptor, wherein detectable binding indicates the presence of an allelic form of the serotonin 5HT_{2C} receptor. Preferably, the cells are human, and may be brain cells. It is preferred that the monoclonal or polyclonal antibody is specific for the extracellular loop of the first transmembrane domain of the serotonin 5HT_{2C} receptor. Labeled antibody is particularly useful, including radiolabeled antibody and fluorescent antibody. With fluorescent antibody, the detecting step can comprise fluorescence activated cell sorting.

The present invention also includes a method for detecting the presence of an allelic variant of the serotonin 5HT_{2C} receptor by 1) providing a biological sample containing the serotonin 5HT_{2C} receptor; 2) contacting the sample with an antibody which specifically binds to a serotonin 5HT_{2C} receptor having a cysteine residue located

at amino acid position 23, and wherein said antibody does not bind a 5HT_{2C} receptor having a serine residue at position 23; 3) detecting the absence of binding of said antibody to said receptor, wherein lack of detectable binding indicates the presence of an allelic form of the serotonin 5HT_{2C} receptor.

Detailed Description of the Invention

5 We describe identification of a non-conservative amino acid substitution in the 5-HT_{2C} receptor gene. The variant was found using single strand conformational polymorphism (SSCP) analysis. By typing this polymorphism in CEPH families, the gene was genetically mapped on the long arm of the X chromosome. Since this polymorphism was not detectable as a conventional RFLP, we also developed a PCR-RFLP based method which allows rapid genotyping in populations and families.

10 Central to the present invention is the discovery of an allelic variant of the serotonin 5HT_{2C} receptor that is functionally different from the predominant wild-type receptor. In this regard, we have discovered an allelic variant of the 5-HT_{2C} receptor. This variant is identical to the wildtype 5-HT_{2C} receptor except for a non-conservative amino acid change in the extracellular portion of the first putative transmembrane domain. A substitution of a serine in place of the normal cysteine occurred in the 23rd amino acid of the final protein. A guanine (G) to cytosine (C)
15 genetic transversion caused a cysteine to be replaced by serine at amino acid 23. As this change places a hydrophilic amino acid in a hydrophobic domain (see FIGURE 1), it would be expected to alter the configuration of the membrane-bound receptor.

Previously, single amino acid substitutions within the 2nd, 3th, 5th, and 7th transmembrane domains of various serotonin receptors have been shown to drastically change the ligand binding characteristics (Baldwin, *Curr. Opin. Cell Biol.* 6:180, 1994; Chanda et al., *Mol. Pharmacol.* 43:516, 1993; Choudhary et al., *Mol. Pharmacol.* 43:755, 1993). In addition, the cysteine at position 23 may be linked to another cysteine residue in the wildtype protein forming a di-sulphide bond bridge. This possibility further suggests that the variant protein, designated 5-HT_{2Cser}, is conformationally and functionally different from the wildtype protein.

20 The present invention includes our discovery of the 5-HT_{2Cser} gene (SEQ ID NO:1) along with the corresponding variant protein product (SEQ ID NO:2) of this gene. In addition, antibodies that can distinguish between the two 5-HT_{2C} forms are anticipated.

One of ordinary skill in the art will appreciate that a kit can be produced that contains all the necessary components to identify a carrier of the HT_{2Cser} gene. This kit would include PCR primers, PCR enzymes, restriction enzymes and any other component useful for determining the one base pair change between the wildtype gene and
30 the allelic variant. PCR primers could be those exemplified below and in SEQ ID NOS: 3 and 4, or constructed by those of ordinary skill in the art as described below.

It should also be appreciated that one could identify the nucleic acid change between the wildtype and the variant 5-HT_{2C} receptor by well known hybridization techniques. Under known conditions, a one base pair mismatch can be determined by, for example, Southern blot analysis or *in situ* hybridization.

Through chromosomal mapping studies we have discovered that 5-HT_{2Cser} is located on the X-chromosome (see FIGURE 5). It is important to recognize that since 5-HT_{2Cser} is located on the X-chromosome, any associated diseases should have an X-linked component.

We identified the 5-HT_{2Cser} polymorphism by amplifying genomic DNA from 62 individuals and performing single strand conformational polymorphism (SSCP) analysis. This procedure is a rapid and efficacious method for detecting polymorphisms (Dean et al, *Cell* 61:863, 1990; Glavac and Dean, *Hum. Mutation* 2:404, 1993; Podusko et al, *Am. J. Hum. Genet.* 49:106, 1992). Although 90% of the 5-HT_{2C} receptor coding region was screened for sequence variants, no other variants were detected. We found that the 5-HT_{2Cser} variant was a relatively common non-conservative amino acid substitution. In a random population sample, the allele frequency of the 5-HT_{2Cser} gene was 13 percent.

Because the polymorphism was not detectable as a conventional restriction fragment length polymorphism (RFLP), we used a PCR primer which introduced a base substitution close to the codon of interest to create an artificial HinfI restriction site in only one of the two allelic forms (Haliassos et al, *Nuc. Acid Res.* 17:3606, 1989). This protocol is discussed in more detail below. Digestion with HinfI yielded two fragments (18 bp and 86 bp) with the 5-HT_{2Cser} gene but left the DNA product undigested (104 bp) with the wildtype 5-HT_{2Ccys} gene. This method is known as the PCR-RFLP method.

As both the SSCP and PCR-RFLP methods can detect the single nucleotide difference between the two serotonin receptor alleles, both methods will yield identical results when genotyping any particular individual. Genotyping 50 individuals using both the PCR-RFLP and SSCP analyses yielded identical results, demonstrating reproducibility of these methods. The conversion of the SSCP-detected variant to a RFLP site facilitates genotyping, makes the genotyping more accessible to a variety of laboratories (eg., research and clinical diagnostic laboratories), and confirms the sequencing results (see below). This is especially important when PCR products are directly sequenced using cycle sequencing.

A tight linkage was found between the described 5-HT_{2C} receptor polymorphism and a marker located on the human X chromosome, DXS734. The DXS734 marker has previously been placed in the Xq21.1 region. Also, positive lod-scores were obtained using a number of other markers in the Xq21.1 region (see TABLE 1).

The Xq21.1 region is interesting because it contains a number of genes that have been correlated with clinical conditions manifested with mental retardation. These diseases include choroideremia, Allan-Herndon-Dudley syndrome, Miles-Carpenter syndromes, MRX7 and MRX8. Therefore, this 5-HT_{2C} receptor is a potential candidate for these syndromes.

The present invention, thus, includes isolated or purified 5-HT_{2Cser} receptor; DNA encoding that receptor; antibodies with specific binding to the 5-HT_{2Cser} receptor and assays for detecting the receptor. In addition, expression vectors encoding 5-HT_{2Cser} and cells expressing the recombinant expression vector are also anticipated. Further, kits and assays for determining the presence or absence of the variant receptor are a part of the present invention. There is an established need for assays useful in correlating behavioral patterns with chemical differences in individuals, and such an assay for the present 5HT variant will be a valuable tool in neuropsychiatric investigations.

The isolated or purified protein is useful, *inter alia*, in competitive assays and in preparation of monoclonal and polyclonal antibody against the variant receptor. An additional facet of the present invention is provision of assays for compounds that bind or block binding to the 5-HT_{2Cser} receptor. Each of these embodiments is discussed in more detail in the following examples.

5 As discussed above, a strong correlation exists between the serotonin family of receptors and some neuropsychiatric diseases. For this reason it is important to identify as many of the serotonin receptor variants as possible. Investigators looking for drugs that affect the quantity of serotonin byproducts need to have methods for testing their candidates against all serotonin receptors. Thus, the 5-HT_{2Cser} variant and associated DNA and assays provide important investigative tools for both behavioral research and the screening of neuropsychiatric drug

10 candidates.

Another application of our discovery is screening individual carriers of the variant gene. Correlations between the allelic variant and neuropsychiatric disorders lead to the necessity of providing screening techniques for identifying carriers of the variant allele.

As a first step we analyzed the 5-HT_{2C} receptor gene for variants in a population of alcoholics.

EXAMPLE 1

Detection of the 5-HT_{2Cser} Variant Allele in Violent Alcoholics Using the Single-Strand Conformation Polymorphism (SSCP) Method

Cell lines were derived from individual patients by immortalizing lymphoblastoid cells with Epstein Barr virus. The populations included Finnish alcoholic violent offenders (n=18), Finnish alcoholic arsonists (n=5) with low CSF 5-HIAA concentrations, alcoholic American Indians (Jamez Pueblo n=8, Cheyenne n=2) and U.S. alcoholic male Caucasians (n=16). DNA samples were taken from these immortalized cell lines using standard protocols. Each genomic DNA sample was amplified in the exon 2 portion of the 5-HT_{2C} receptor gene by using the polymerase chain reaction (PCR) with primers that border the exon 2 region: 5HT2C7 (5' CACCTAATTGGCCTATTGGTT 3') (SEQ ID NO:3) and 5HT2C8 (5' AAGGATTGCCAGGAGAGACAG 3') (SEQ ID NO:4). Using these two primers will allow amplification of the region encoding exon 2 of the gene. Although these primers were used to amplify the region of interest, it will be appreciated by those skilled in the art that other primers could accomplish the same result.

Amplification was performed in 7.5 μ l reactions consisting of 30 ng of DNA, 0.25 μ M of each primer, 250 μ M each of dATP, dCTP, dGTP, dTTP, 1 μ Cl of (α ³²P) dCTP, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 10 mM Tris-HCl (pH 8.3) and 0.75 units of Taq DNA polymerase (AmpliTaQ from Perkin Elmer Cetus).

30 DNA samples were amplified for 30 cycles, each consisting of 1 min at 95°C, 2 min at 55°C and 3 min at 72°C. After amplification, the radioactively labeled amplified DNA was digested with 5 units of restriction enzyme Rsa I under standard conditions to allow this enzyme to generate smaller DNA fragments to increase the sensitivity of SSCP detection.

35 Following digestion, the DNA was denatured by diluting the reaction mixture with 15 μ l of 95% formamide, 10 mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue, and incubating at 95°C for 3 min followed by chilling on ice.

We loaded 4 μ l of each denatured DNA sample onto a Mutation Detection Enhancement (MDE) gel (AT Biochem, Malvern, PA). The DNA was electrophoresed at 4°C for 16 hr at 6 V. MDE gels contain polyvinyl and are suggested to be superior to acrylamide gels in sensitivity for detecting small mutational differences between DNA sequences. The MBE gel was dried and autoradiography was performed at -70°C. DNA fragments containing a single nucleotide difference were detected by their different relative rates of electrophoretic migration on this gel.

To provide an additional assay for determining carriers of the 5-HT_{2Cser} variant, we performed nucleic acid sequencing reactions of the amplified PCR fragments described above. However, one of ordinary skill in the art will appreciate that many ways are available to directly determine the nucleic acid sequence of DNA fragments.

EXAMPLE 2

DNA Sequence Determination of PCR DNA Products to Identify the 5-HT_{2Cser} Variant Allele

The PCR product was purified by agarose gel electrophoresis followed by extraction with glass beads (Geneclean, BIO 101, La Jolla, CA). DNA was directly sequenced by dideoxy cycle-sequencing according to the manufacturers' instructions (Life Technologies, Gaithersburg, MD).

A G to C transversion caused a cysteine (encoded by TGT) to be replaced by serine (encoded by TCT) at amino acid position 23. These sequences were determined from two males with the 5-HT_{2Ccys} genotype, one male with the 5-HT_{2Cser} genotype and a female who is heterozygous for the 5-HT_{2Ccys}/5-HT_{2Cser} genotypes.

The sequence establishes the molecular basis for the genetic polymorphism detected using the methods described in Example 1. Furthermore, the amino acid substitution encoded by the variant DNA sequence establishes that the 5-HT_{2C} variant receptor protein has a serine at position 23 which may be physiologically significant. Also, the particular DNA sequence of the variant 5-HT_{2Cser} allele facilitated development of another, technically easier assay, called PCR-RFLP (described in Example 4), for determining the genotype of different individuals.

Having discovered the polymorphism, we then determined the chromosomal location of this gene as described below in Example 3.

EXAMPLE 3

Genetic Mapping of the 5-HT_{2C} Receptor (HTR2C)

The 5-HT_{2C} genotype was determined in 10 informative CEPH families. Data were entered into programs provided by CEPH, and files with X chromosome markers were prepared. Two-point lod score analysis was performed using LINKAGE (Lathrop et al., *Proc. Natl. Acad. Sci. USA* 81:3443, 1984) and MAPMAKER (Lander et al., *Genomics* 1:174, 1987), and the two-point values were utilized for multipoint analysis. Multipoint linkage analysis was performed using markers that showed the lowest recombination fraction. Also, other highly informative markers were chosen from both the Genethon (Weissenbach et al., *Nature* 359:794, 1992) and NIH/CEPH consortium maps (*Science* 258:87, 1992). The 5-HT_{2C} gene was placed in all positions of a map of markers ordered with greater than 1000:1 odds, using the TRY command of MAPMAKER. The data analyzed in this manner for a number of genetic markers is presented in TABLE 1.

The 5-HT_{2C} receptor polymorphism was found to segregate in an X-linked codominant fashion in CEPH families and the genotypes for 10 informative families were determined. Allele frequencies in 47 unrelated caucasians (CEPH parents) were 0.13 and 0.87 for 5-HT_{2C}ser and 5-HT_{2C}cys, respectively. Using these same families, the gene was mapped by using two-point lod scores (TABLE 1 shows the maximum scores). Two-point analysis yielded a maximum lod-score of 10.22 to DXS734 which has previously been assigned to Xq21.1. There were also a number of other markers in this region which were linked to the 5-HT_{2C} receptor gene. Multipoint analysis placed the 5-HT_{2C} gene on the long arm of X chromosome and in the interval between DXS362 and DXS42 with odds greater than 1000:1. Sex averaged intervals between the 5-HT_{2C} gene locus and markers are shown in FIGURE 2.

Table 1. Markers linked to HTR2C

	Locus	Probe	Enzyme	Location	<i>q</i>	Z _{max}
10	DXS1001	248we5	(AC) _n	X	8.6	3.67
		454	GT	Xq	18.0	3.05
		458	GT	Xq	18.0	3.51
	DXS329	K20-7	HindIII	Xq24-q26	6.2	3.19
15	DXS328	OST-13	HindIII	Xq21.3-q22.1	12.2	5.14
	DXS328	OST-47	HindIII	Xq21.3-q22.1	11.6	3.49
	DXS327	OST-7	MspI	Xq21.2-q22.1	5.0	3.69
	DXS350	RX-100M1	MspI	Xq21.1-q24	0.0	4.51
	DXS350	RX-100M2	MspI	Xq21.1-q24	0.0	5.71
20	DXS358	RX-187	HindIII	Xq24-qter	0.0	7.22
	DXS358	RX-187A	TaqI	Xq24-qter	0.0	6.01
	DXS358	RX-187B	TaqI	Xq24-qter	0.0	4.21
	DXS362	RX-237	MspI	Xq21.1-qter	2.7	8.24
	DXS362	RX-237	TaqI	Xq21.1-qter	8.1	6.01
25	DXS368	RX-329	TaqI	Xq21.1-q24	0.0	3.31
	DXS447	RX-404	BglII	Xq21.1	13.8	3.38
	DXS734	RX-99	TaqI	Xq21.1	0.0	10.22
	DXS456	XG30B	PCR	Xq21-q22	7.5	4.73
		ch26	HindIII	X	14.2	4.56
30	DXS3	P19-2	MspI	Xq21.3	9.5	3.15

The DXS markers are anonymous loci. "Enzyme" refers to the enzyme that detects the polymorphism; "(AC)_n" and "GT" represent CA/GT dinucleotide repeat loci; "Location" is the known physical location of the marker on the X chromosome. "*q*" is the sex-averaged recombination frequency observed with 5HT_{2C} and "Z_{max}" is the LOD (log of odds) score at the value of the recombination frequency.

These results mapped the 5-HT_{2C} receptor gene to a small portion of the long arm of the X chromosome which is genetically linked to a variety of known mental retardation syndromes such as choroideremia, Allan-Hammond-Dudley syndrome, Moles-Carpenter syndromes, MRX7 and MRX8. Thus, the described 5-HT_{2C} genetic polymorphism could be useful in diagnosis of mental retardation syndromes.

We also developed a technically easier assay, called PCR-RFLP, for determining the genotype of different individuals.

EXAMPLE 4

Detection of the 5-HT_{2C}ser Variant Allele in Individuals by Using the PCR-RFLP Method

A 104 bp region of the 5-HT_{2C} receptor gene near the polymorphism was amplified using a second set of PCR primers. One of the PCR primers was used to introduce a base substitution close to the codon which differs in the 5-HT_{2C}ser variant allele, creating an artificial *Hinf*I restriction site only in the amplified DNA corresponding to the 5-HT_{2C}ser variant allele (Hadjassos et al., *Nuc. Acid Res.* 17:3606, 1989).

The PCR primers were REPA1 (5' TTG6CCTATTGGTTTGGGAAT 3') (SEQ ID NO:5) and ARTIFACT2 (5' GTCTGGGAATTTGAAGCGTCCAC 3') (SEQ ID NO:6). REPA1 introduces a C to G substitution 4 bp upstream from the polymorphic site. This substitution artificially creates a *Hinf*I RFLP. That is, when DNA containing the 5HT_{2C}ser allele is amplified using these primers and then digested with *Hinf*I, it yields two fragments (18 bp and 86 bp). These fragments can be detected by commonly used gel electrophoresis.

In contrast, when DNA containing the more common 5-HT_{2C}cys allele was similarly amplified and treated with *Hinf*I, the amplification product remained undigested and was seen as a single band (104 bp) following gel electrophoresis. Amplification products were visualized in ethidium bromide stained 10% polyacrylamide gels. In this study, we amplified the DNA from a population including a total of 50 controls and patients with psychiatric disorders.

Amplification was performed using 60 ng of DNA, 0.25 μ M of each primer, 250 μ M each of dATP, dCTP, dGTP, dTTP, 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 10mM Tris-HCl (pH 8.3) and 0.75 units of AmpliTaq (Perkin Elmer Cetus) in a total volume of 15 μ L. Samples were amplified for 35 cycles, each consisting of 1 min at 95°C, 2 min at 50°C 3 min at 72°C.

After amplification, 10 units of *Hinf*I (New England Biolabs) and recommended buffer (1 x concentration with respect to the final volume) were added directly to PCR samples and incubated for 10 h at 37°C. The DNA fragments were resolved by 10% polyacrylamide gel electrophoresis and stained with ethidium bromide.

To check the accuracy of the PCR-RFLP method, we performed the SSCP method on the same 50 people, including patients and controls, as those used for the PCR-RFLP experiment. We found that both the SSCP method and the PCR-RFLP method detected the same allelic variant in the 5-HT_{2C} receptor gene. For this reason, both methods should be equally reliable in typing individuals for their respective 5-HT_{2C} receptor allele(s). Note that because the 5-HT_{2C} receptor gene is located on the X chromosome (see Example 3), generally males will carry only one allele. However, females can be either homozygous or heterozygous for these two alleles.

The PCR-RFLP method is technically easier than the SSCP method because it does not require radioactive labeling of the amplified DNA. In the PCR-RFLP method, amplified DNA fragments are detected by electrophoresis through a gel without requiring denaturation of the DNA. Thus the PCR-RFLP method allows rapid genotyping of populations and families.

Having determined the relative frequencies of the two identified forms of the 5-HT_{2C} receptor in a normal population using the PCR-RFLP method, we determined whether these alleles occur at abnormal frequencies in subpopulations exhibiting several neuropsychiatric disorders.

EXAMPLE 5

Detection of the 5-HT_{2Cser} Variant Allele in Finnish Alcoholic Violent Offenders

Using the SSCP and PCR-RFLP methods described above, we isolated DNA from immortalized cells derived from Finnish alcoholic violent individuals. Subjects were further classified whether they fulfilled the DSM III criteria for antisocial personality disorder (APD), intermittent explosive disorder (IED), or other DSM III diagnoses. In addition, we took DNA from cell lines of controls that had been psychologically interviewed and determined to be mentally healthy. TABLE 2 shows the results of this experiment.

TABLE 2.

5-HT_{2C} Genotype Frequencies in Alcoholic Violent Offenders and Controls

	5-HT _{2C} cys	5-HT _{2C} ser	# Subjects
ASP	0.88	0.14	72
IED	0.92	0.08	38
Other offenders	0.95	0.05	37
All offenders	0.90	0.10	147
Controls	0.82	0.18	123

To determine if the 5-HT_{2Cser} variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation exhibiting suicidal behavior.

EXAMPLE 6

Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Suicidal Behavior by Using the SSCP Method

Using the SSCP method described in Example 1, genomic DNA isolated from suicidal individuals is analyzed for the frequency of the 5-HT_{2Cser} allele. Because suicidal behavior has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting suicidal tendencies or behavior compared

to a normal population. A correlation is found between the patients carrying the allelic variant of the 5-HT_{2C} receptor and individuals exhibiting suicidal behavior.

To determine if the 5-HT_{2C}ser variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using subpopulations exhibiting panic disorder and clinical anxiety.

EXAMPLE 7

Detection of the 5-HT_{2C}ser Variant Allele in Individuals Exhibiting Panic Disorder and/or Anxiety by Using the SSCP Method

Using the SSCP method described in Example 1, genomic DNA isolated from individuals exhibiting panic disorder or clinical anxiety is analyzed for the frequency of the 5-HT_{2C}ser allele. Because panic disorder and clinical anxiety have been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting panic disorder and/or anxiety compared to a normal population. A correlation is found between the patients carrying the allelic variant of the 5-HT_{2C} receptor and individuals exhibiting panic disorder and/or clinical anxiety.

To determine if the 5-HT_{2C}ser variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation exhibiting seasonal affective disorder.

EXAMPLE 8

Detection of the 5-HT_{2C}ser Variant Allele in Individuals Exhibiting Seasonal Affective Disorder by Using the SSCP Method

Using the SSCP method described in Example 1, genomic DNA isolated from individuals exhibiting seasonal affective disorder is analyzed for the frequency of the 5-HT_{2C}ser allele. Because seasonal affective disorder has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting seasonal affective disorder compared to a normal population. A correlation is found between the patients carrying the allelic variant of the 5-HT_{2C} receptor and individuals exhibiting seasonal affective disorder.

To determine if the 5-HT_{2C}ser variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation exhibiting obsessive-compulsive disorder.

EXAMPLE 9

Detection of the 5-HT_{2C}ser Variant Allele in Individuals Exhibiting Obsessive-Compulsive Disorder by Using the SSCP Method

Using the SSCP method described in Example 1, genomic DNA isolated from individuals exhibiting obsessive-compulsive disorder is analyzed for the frequency of the 5-HT_{2C}ser allele. Because obsessive-compulsive disorder has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting obsessive-compulsive disorder compared to a normal population. To confirm the results obtained using the SSCP method, the PCR-RFLP method is also used on the sample from each individual. To determine if the 5-HT_{2C}ser variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation having eating disorders.

EXAMPLE 10**Detection of the 5-HT_{2Cser} Variant Allele in Individuals with Eating Disorders by Using the PCR-RFLP Method**

5 Using the PCR-RFLP method described above, genomic DNA isolated from individuals with eating disorders is analyzed for the frequency of the 5-HT_{2Cser} allele. Because eating disorders have been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting eating disorders compared to a normal population.

To determine if the 5-HT_{2Cser} variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation exhibiting schizophrenia.

EXAMPLE 11**Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Schizophrenia by Using the PCR-RFLP Method**

15 Using the PCR-RFLP method described above, genomic DNA isolated from schizophrenic individuals is analyzed for the frequency of the 5-HT_{2Cser} allele. Because schizophrenic behavior has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting schizophrenic tendencies or behavior compared to a normal population.

To determine if the 5-HT_{2Cser} variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using subpopulations exhibiting myoclonic disorders.

EXAMPLE 12**Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Myoclonic Disorders by Using the PCR-RFLP Method**

25 Using the PCR-RFLP method described above, genomic DNA isolated from individuals exhibiting myoclonic disorder is analyzed for the frequency of the 5-HT_{2Cser} allele. Because myoclonic disorder has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting myoclonic disorder compared to a normal population.

To determine if the 5-HT_{2Cser} variant allele is linked to other neuropsychiatric disorders, a similar analysis is done using a subpopulation with epilepsy.

EXAMPLE 13**Detection of the 5-HT_{2Cser} Variant Allele in Individuals with Epilepsy by Using the PCR-RFLP Method**

35 Using the PCR-RFLP method described above, genomic DNA isolated from individuals having epilepsy is analyzed for the frequency of the 5-HT_{2Cser} allele. Because epilepsy has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting epilepsy compared to a normal population.

To determine if the 5-HT_{2Cser} variant allele is linked to other disorders, a similar analysis is done using a subpopulation exhibiting manic-depressive illness.

EXAMPLE 14**Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Manic-Depressive Illness by Using the PCR-RFLP Method**

Using the PCR-RFLP method described above, genomic DNA isolated from individuals exhibiting manic-depressive illness is analyzed for the frequency of the 5-HT_{2Cser} allele. Because manic-depressive illness has been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting manic-depressive illness compared to a normal population.

EXAMPLE 15**Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Type I or Type II Alcoholism by Using the PCR-RFLP Method**

Using the PCR-RFLP method described above, genomic DNA isolated from individuals exhibiting Type I or Type II alcoholism is analyzed for the frequency of the 5-HT_{2Cser} allele. Because Type I and Type II alcoholism have been shown to have a genetic component, the frequency of this allele is determined in the subpopulation exhibiting Type I or Type II alcoholism compared to a normal population.

EXAMPLE 16**Detection of the 5-HT_{2Cser} Variant Allele in Individuals Exhibiting Mental Retardation Associated with the Xq21.1 Chromosomal Region by Using the PCR-RFLP Method**

The Xq21.1 region contains a number of genes that have been correlated with clinical conditions manifested with mental retardation including choroideremia, Allan-Herndon-Dudley syndrome, Miles-Carpenter syndromes, MRX7 and MRX8. Using the PCR-RFLP method described above, genomic DNA is isolated from individuals exhibiting one of these forms of mental retardation and analyzed for the frequency of the 5-HT_{2Cser} allele. The frequency of this allele is determined in the subpopulation exhibiting Xq21.1-linked mental retardation compared to a normal population.

EXAMPLE 17**Production of Antibodies Against the 5-HT_{2Cser} Variant Receptor Protein**

Cells expressing the 5-HT_{2Cser} variant receptor are obtained from human CSF and lysed with NP40, and the isolated membranes are injected into rabbits. The lysed membranes are isolated in a non-ionic detergent so as not to affect the membrane bound receptors. Freund's adjuvant is used in the injection to help stimulate an antigenic response by the rabbits using well known methods. After two booster shots of the lysed membranes, the rabbits are bled and the sera isolated by centrifugation.

The antibodies in the crude rabbit sera extract are ¹²⁵I labeled by well-known methods and tested for activity against transformed COS7 cells expressing the variant receptor. COS7 cells expressing the wildtype allele are used to preabsorb antibodies with nonspecific binding. A Western blot having one lane containing protein lysates

from COS7 cells expressing the variant gene, and a second lane having lysates from COS7 cells expressing the wildtype 5-HT_{2Cys} receptor (control) is run.

Monoclonal antibodies can be made by well known methods in addition to the polyclonal antibodies discussed above. One method of producing monoclonal antibodies is discussed below.

5 These antibodies will specifically recognize the variant 5-HT_{2Cser} receptor protein on cell membranes. Antibodies of this type can be used as research tools to characterize expression of this variant on different cells or in different populations or under different physiological conditions. Antibodies can further be used as a diagnostic of abnormal conditions linked to expression of the variant 5-HT_{2Cser} receptor.

10 EXAMPLE 18

Production of Monoclonal Antibodies Against the 5-HT_{2Cser} Variant Receptor Protein

Cells expressing the 5-HT_{2Cser} receptor are isolated from human CSF and lysed with NP40. The cell membranes are pelleted by centrifugation and isolated membranes having bound 5-HT_{2Cser} variant receptor proteins are injected in Freund's adjuvant into mice. After being injected 9 times over a three week period, the mice spleens are removed and following conventional cell separation techniques were resuspended in PBS.

15 The suspended spleen cells are mixed (approximately 4:1) with SP 2/D Myeloma cells. Polyethylene glycol is added to fuse the myeloma cells to the spleen cells, and the fused cells are selected in HAT media. The fused cells are aliquoted so that only one cell is grown in each well of a 96-well microtiter plate. Each cell is grown, the media removed and secreted proteins in the media are ¹²⁵I or fluorescently labeled. The labeled media from each well is used to probe a Western blot of cell lysates having the 5-HT_{2Cser} variant and the 5-HT_{2Cys} receptor. The desired fusion cell will produce a monoclonal antibody that strongly binds the 5-HT_{2Cser} variant receptor lane on the Western blot, but doesn't bind to a similar sized protein in the 5-HT_{2Cys} (control) lane.

20 It will be appreciated by those skilled in the art that a monoclonal antibody that specifically recognizes the 5-HT_{2Cys} form of the receptor but does not recognize the variant 5-HT_{2Cser} receptor could also be used for detection of presence of the variant. That is, such a monoclonal antibody would give a negative response with receptors from a homozygotic person for the 5-HT_{2Cser} allele while giving a positive response with a 5-HT_{2Cys} control.

25 These monoclonal antibodies provide a way of detecting expression of the 5-HT_{2Cser} variant serotonin receptor protein. Another method of detecting expression of the 5-HT_{2Cser} variant is by *in situ* hybridization.

EXAMPLE 19

In Situ Hybridization Using 5-HT_{2Cser} Variant Gene Fragments

35 *In situ* hybridization allows the identification of mRNA within intact tissues, including from human brain biopsies. In this method, oligonucleotides corresponding to a portion of the 5-HT_{2Cser} variant gene (SEQ ID NO: 1) are used to detect specific mRNA species in the brain.

Biopsied brain tissue is perfused with a 4% formaldehyde solution using standard histology methods. The brain tissue is frozen with liquid nitrogen and cut into 5 μ m to 30 μ m sections that are placed on slides and incubated in proteinase K for approximately 15 minutes. The slides are then rinsed in diethylpyrocarbonate-treated water, and ethanol, and placed in a prehybridization buffer.

5 A radioactive probe corresponding to the PCR product obtained from using primers SEQ ID NO:3 and SEQ ID NO:4 (see Example 1) is made by performing the PCR using [α - 32 P]dCTP in the reaction mixture. The labeled PCR product is incubated with the sectioned brain tissue using standard hybridization methods. After incubation and washing at a temperature that allows binding to the 5-HT_{2Cser} variant mRNA but not to the 5-HT_{2Ccys} mRNA, the slides are air dried and labeled areas are visualized by autoradiography. Dark spots on the tissue sample indicate hybridization of the probe with the brain mRNA thereby demonstrating expression of the 5-HT_{2Cser} variant receptor.

EXAMPLE 20

Site-directed Mutagenesis and Synthesis of mRNA

15 A human 5-HT_{2C} receptor cDNA clone consisting of the entire coding region of the 5-HT_{2C} receptor (Saltzman et al., *Biochem. Biophys. Res. Commun.* 181:1489, 1991) was subcloned into an pSP72 vector at EcoRI and XbaII sites for mutagenesis. The single-base mutation that converts cysteine (encoded by TGT) to serine (encoded by TCT) in the resulting protein was introduced into the human 5-HT_{2C} receptor cDNA at codon 23 by recombinant PCR (Vallette et al., *Nuc. Acid Res.* 17:723, 1989). The authenticity of the single base mutation was confirmed by double stranded DNA sequencing (United States Biochemical, Cleveland, OH). Complementary RNA was prepared by in vitro transcription using the mCAP™ kit from Stratagene (La Jolla, CA).

20 The mutated cDNA provided a ready source of mRNA specific for the 5-HT_{2Cser} receptor. That mRNA as well as mRNA prepared from the cDNA encoding the 5-HT_{2Ccys} receptor was expressed in oocytes isolated from the African clawed toad, *Xenopus laevis*. Following expression, the oocytes were tested for differences in ligand binding between the wildtype and allelic variant of the 5-HT_{2C} receptor present on the oocytes' surface.

EXAMPLE 21

Oocyte Preparation, Oocyte Expression and Pharmacological Characterization of 5-HT_{2Ccys} and 5-HT_{2Cser} Using Electrophysiological Recording

30 The oocytes were isolated from mature female *Xenopus laevis*. The follicular layer of the oocytes was removed by treatment with 0.2% collagenase A, and oocytes were transferred into a modified Barth's saline (MBS) solution containing: NaCl 88 mM, KCl 1 mM, NaHCO₃ 2.4 mM, Ca(NO₃)₂ 0.3 mM, CaCl₂ 0.9 mM, MgSO₄ 0.8 mM, HEPES 10 mM (pH 7.5), to await mRNA or cRNA injection. Approximately 16-20 ng cRNA was injected per oocyte using a microinjection pump (World Precision Instruments, New Haven, CT). Between two and three days following oocyte cRNA microinjection, whole cell currents were measured in a perfusion medium MBS under two-electrode voltage-clamp at -70 mV. Serotonin was superfused at the rate of approximately 3 ml/min. for 30 seconds, with a period of 20 minutes between applications.

Xenopus oocytes have endogenous Ca^{2+} -activated chloride channels that can serve as a functional indicator for receptor-mediated inositoltriphosphate (IP3) increase. Taking advantage of this property, the oocyte expression system has been widely used to pharmacologically characterize 5-HT_{2C} function after microinjection of the synthetic receptor mRNA into oocytes (Julius et al., *Science* 241:558, 1988). We compared the electrophysiological properties of the human 5-HT_{2Ccys} and 5-HT_{2Cser} under identical conditions in oocytes expressing these receptors. As shown in FIGURE 3, serotonin (5-HT) activated similar inward oscillating currents with a slow onset (3-10 sec) in oocytes expressing either 5-HT_{2Cser} or 5-HT_{2Ccys}.

In agreement with others, we found that by allowing at least 20 min between the drug applications it was possible to achieve consistency in subsequent 5-HT-activated responses. The currents activated by 10 nM 5-HT were suppressed in the presence of 1 μ M 1-(1-naphthyl) piperazine HCL (PPRZ), which is a selective antagonist for 5-HT_{2A} and 5-HT_{2C} receptors. The effects of PPRZ on 5-HT-activated response for both types of receptors was reversible after a 20 min wash. A detectable inward current was activated by 1 nM 5-HT in cells expressing either 5-HT_{2Ccys} or 5-HT_{2Cser} receptors. The amplitude of 5-HT-activated currents increased in a concentration-dependent manner as agonist concentrations were increased from 1-500 nM (see FIGURE 4). Similar maximal responses were obtained with 500 nM 5-HT in oocytes expressing either the 5-HT_{2Ccys} or 5-HT_{2Cser}.

EXAMPLE 22

Ligand Binding to 5-HT_{2Ccys} and 5-HT_{2Cser} Receptors in Human Cells

Cells expressing the 5-HT_{2Ccys} or 5-HT_{2Cser} receptors are isolated from human tissue, including from brain biopsies or cells isolated from CSF, using commonly known methods. The cells are incubated with known or suspected ligands for sufficient time and in sufficient concentrations to allow binding to the 5-HT_{2C} receptors, employing commonly used methods for determining binding *in vitro*. Known or suspected ligands include 5-HT analogs, 5-HT antagonists, and various pharmacological drugs used to treat neuropsychiatric disorders. Ligands are labeled either radioactively or fluorescently and relative binding of the ligand to the 5-HT_{2C} receptors is measured by measuring the amount of radioactivity or fluorescence attached to the membranes after the unbound fraction is washed away.

The relative binding efficiencies of ligands may be useful in developing new drugs to treat certain clinical conditions, or in determining effective drug dosages for clinical treatment of individuals with specific genotypes for the 5-HT_{2C} receptor.

EXAMPLE 23

Transfection of Mammalian Cells with the 5-HT_{2Ccys} and 5-HT_{2Cser} Receptor Genes

Expression of the two forms of the 5-HT_{2C} serotonin receptor is assayed in mammalian COS7 cells transfected with the genes encoding the 5-HT_{2Ccys} or 5-HT_{2Cser} receptor proteins in an expression plasmid, pSR α . The 5-HT_{2Ccys} and 5-HT_{2Cser} genes individually are subcloned into the expression vector pSR α by using standard

molecular cloning methods. The transfected COS7 cells are then placed in media for about 72 hours to allow expression of the 5-HT_{2C}cys or 5-HT_{2C}ser receptors on their cell surface.

COS7 cells are transiently transfected with the pSR α -5-HT_{2C}cys or pSR α -5-HT_{2C}ser construct using the calcium phosphate precipitation method as previously described by Monsma, F.J. et al., *Proc. Natl. Acad. Sci. USA* 87:6723, 1990. Cells are harvested 72 hours after transfection and either used directly or the membranes containing the 5-HT_{2C} receptors are isolated from the cells using methods described below.

Cells or membranes isolated from them are used to assay binding of various ligands of pharmacological interest. The relative binding efficiencies of ligands are useful in developing new drugs to treat clinical conditions associated with 5-HT_{2C} receptors.

While COS7 cells are a preferred embodiment, those skilled in the art will appreciate that other cells and vectors could be used in transfection for expression of the 5-HT_{2C} receptor genes.

EXAMPLE 24

Ligand Binding to Mammalian Cells Transfected with the 5-HT_{2C}cys and 5-HT_{2C}ser Receptor Genes

Mammalian COS7 cells are transfected as described above, and binding of ligands to the 5-HT_{2C}cys or 5-HT_{2C}ser serotonin receptors is assayed using whole cells expressing the 5-HT_{2C}cys or 5-HT_{2C}ser receptors on their surface.

Cells are collected 72 hours after transfection and placed in fresh media containing radiolabeled or fluorescently labeled ligands. Ligands include 5-HT analogs, 5-HT antagonists, and various pharmacological drugs used to treat neuropsychiatric disorders. After incubation for various times to allow binding of the ligands to the 5-HT_{2C} receptors, cells are washed and then assayed for binding of the ligand to the cells, employing commonly used methods for detecting radioactivity or fluorescence.

This method is useful because cells that express 5-HT_{2C} receptors can be obtained with relative ease without relying on clinical samples. Mammalian COS7 cells probably reflect the physiological conditions normally affecting expression of serotonin receptors in humans. The relative binding efficiencies of ligands are useful in developing new drugs to treat clinical conditions associated with 5-HT_{2C} receptors, or in determining effective drug dosages for clinical treatment of individuals with specific genotypes for the 5-HT_{2C} receptor.

EXAMPLE 25

Ligand Binding to Membranes Isolated from Mammalian Cells Transfected with the 5-HT_{2C}cys and 5-HT_{2C}ser Receptor Genes

Mammalian COS7 cells are transfected as described above, and binding of ligands to the 5-HT_{2C}cys or 5-HT_{2C}ser serotonin receptors is assayed using membranes isolated from cells expressing the 5-HT_{2C}cys or 5-HT_{2C}ser receptors on their surface. Cells are harvested 72 hours after transfection and either disrupted in a dounce homogenizer in 50 mM Tris-HCl, pH 7.4 at 37°C, 10 mM MgSO₄ and 0.5 mM EDTA, or frozen in 5 mM Tris-HCl, pH 7.4 at 25°C, 5 mM MgCl₂, 250 mM sucrose and stored in liquid N₂ prior to membrane preparation. Crude

membranes were prepared from cell homogenates by centrifugation at 43,000 x g. and re-suspension in homogenization buffer at a protein concentration of 80 µg/ml.

5 Once the crude membranes are prepared from the cell homogenates, screening of various pharmacologically indicated ligands for differential binding to the 5-HT_{2C} receptors is performed as discussed above. Ligands, which are radiolabeled or fluorescently labeled, include 5-HT analogs, 5-HT antagonists, and various pharmacological drugs used to treat neuropsychiatric disorders.

10 This method is useful because it provides a ready source of serotonin receptors expressed on mammalian cells for comparison of many different ligands, alone or in combination, under identical binding conditions. The information from the assay relating to relative binding efficiencies of ligands is useful in developing new drugs to treat certain clinical conditions associated with expression of the different forms of the 5-HT_{2C} receptor.

SEQUENCE LISTING

(1) GENERAL INFORMATION

- 5 (i) APPLICANT: United States Government as represented
by the Secretary, Department of Health
and Human Services
- 10 (ii) TITLE OF THE INVENTION: ALLELIC VARIATION OF THE SEROTONIN
5HT2C RECEPTOR
- (iii) NUMBER OF SEQUENCES: 6
- 15 (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Knobbe, Martens, Olson and Bear
(B) STREET: 620 Newport Center Drive 16th Floor
(C) CITY: Newport Beach
20 (D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 92660
- 25 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ Version 1.5
- 30 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- 35 (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/310,271
(B) FILING DATE: 21-SEP-1994
- 40 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Fuller, Michael L
(B) REGISTRATION NUMBER: 36,516
(C) REFERENCE/DOCKET NUMBER: NIH103.001VPC
- 45 (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 619-235-8550
(B) TELEFAX: 619-235-0176
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

- 50 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2733 base pairs
(B) TYPE: nucleic acid
55 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
60 (v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:
(ix) FEATURE:
(A) NAME/KEY: Coding Sequence

(B) LOCATION: 788...2164
(D) OTHER INFORMATION:

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAATTCGGGA GCGTCCTCAG ATGCACCGAT CTTCCCGATA CTGCCTTTGG AGCGGCTAGA 60
 TTGCTAGCCT TGGCTGCTCC ATTGGCCTGC CTGCCCCCTT ACCTGCCGAT TGCATATGAA 120
 CTCTTCTTCT GTCTGTACAT CGTTGTGCTC GGAGTCGTGC CGATCGTCTG GCGCTCGTG 180
 TGATGGCCTT CGTCCGTTTA GAGTAGTGTA GTTAGTTAGG GGCCAACGAA GAAGAAAGAA 240
 GACGCGATTA GTGCAGAGAT GCTGGAGGTG GTCAGTTACT AAGCTAGAGT AAGATAGCGG 300
 AGCGAAAAGA GCCAAACCTA GCCGGGGGGC GCACGGTCAC CCAAAGGAGG TCGACTCGCC 360
 GCGCTTCCT ATCGCGCCGA GCTCCCTCCA TTCCTCTCCC TCCGCCGAGG CGCGAGGTTG 420
 CGCGCGCAG CGCAGCGCAG CTCAGCGCAC CGACTGCCGC GGGCTCCGCT GGGCGATTGC 480
 AGCCGAGTCC GTTCTCGTC TAGCTGCCGC CGCGGCGACC GCTGCCTGGT CTTCTCCCG 540
 GACGCTAGTG GGTATCAGC TAACACCCGC GAGCATCTAT AACATAGGCC AACTGACGCC 600
 ATCCTTCAAA ACAAACCTGTC TGGGAAAAAA AGAATAAAAA GTAGTGTGAG AGCAGAAAAAC 660
 GTGATTGAAA CACGACCAAT CTTTCTTCAG TGCCAAAGGG TGGAAAAGAA AGGATGATAT 720
 GATGAACCTA GCCTGTTAAT TTCGTCTTCT CAATTTTAAA CTTTGGTTGC TTAAGACTGA 780
 AGCAATC ATG GTG AAC CTG AGG AAT GCG GTG CAT TCA TTC CTT GTG CAC 820
 Met Val Asn Leu Arg Asn Ala Val His Ser Phe Leu Val His
 1 5 10
 CTA ATT GGC CTA TTG GTT TGG CAA TCT GAT ATT TCT GTG AGC CCA GTA 877
 Leu Ile Gly Leu Leu Val Trp Gln Ser Asp Ile Ser Val Ser Pro Val
 15 20 25 30
 GCA GCT ATA GTA ACT GAC ATT TTC AAT ACC TCC GAT GGT GGA CGC TTC 925
 Ala Ala Ile Val Thr Asp Ile Phe Asn Thr Ser Asp Gly Gly Arg Phe
 35 40 45
 AAA TTC CCA GAC GGG GTA CAA AAC TGG CCA GCA CTT TCA ATC GTC ATC 973
 Lys Phe Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Ile
 50 55 60
 ATA ATA ATC ATG ACA ATA GGT GGC AAC ATC CTT GTG ATC ATG GCA GTA 1021
 Ile Ile Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val
 65 70 75
 AGC ATG GAA AAG AAA CTG CAC AAT GCC ACC AAT TAC TTC TTA ATG TCC 1069
 Ser Met Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser
 80 85 90
 CTA GCC ATT GCT GAT ATG CTA GTG GGA CTA CTT GTC ATG CCC CTG TCT 1117
 Leu Ala Ile Ala Asp Met Leu Val Gly Leu Leu Val Met Pro Leu Ser
 95 100 105 110
 CTC CTG GCA ATC CTT TAT GAT TAT GTC TGG CCA CTA CCT AGA TAT TTG 1165
 Leu Leu Ala Ile Leu Tyr Asp Tyr Val Trp Pro Leu Pro Arg Tyr Leu
 115 120 125

TGC CCC GTC TGG ATT TCT TTA GAT GTT TTA TTT TCA ACA GCG TCC ATC 1213
Cys Pro Val Trp Ile Ser Leu Asp Val Leu Phe Ser Thr Ala Ser Ile
130 135 140

5 ATG CAC CTC TGC GCT ATA TCG CTG GAT CGG TAT GTA GCA ATA CGT AAT 1261
Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile Arg Asn
145 150 155

10 CCT ATT GAG CAT AGC CGT TTC AAT TCG CGG ACT AAG GCC ATC ATG AAG 1309
Pro Ile Glu His Ser Arg Phe Asn Ser Arg Thr Lys Ala Ile Met Lys
160 165 170

15 ATT GCT ATT GTT TGG GCA ATT TCT ATA GGT GTA TCA GTT CCT ATC CCT 1357
Ile Ala Ile Val Trp Ala Ile Ser Ile Gly Val Ser Val Pro Ile Pro
175 180 185 190

20 GTG ATT GGA CTG AGG GAC GAA GAA AAG GTG TTC GTG AAC AAC ACG ACG 1405
Val Ile Gly Leu Arg Asp Glu Glu Lys Val Phe Val Asn Asn Thr Thr
195 200 205

TGC GTG CTC AAC GAC CCA AAT TTC GTT CTT ATT GGG TCC TTC GTA GCT 1453
Cys Val Leu Asn Asp Pro Asn Phe Val Leu Ile Gly Ser Phe Val Ala
210 215 220

25 TTC TTC ATA CCG CTG ACG ATT ATG GTG ATT ACG TAT TGC CTG ACC ATC 1501
Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu Thr Ile
225 230 235

30 TAC GTT CTG CGC CGA CAA GCT TTG ATG TTA CTG CAC GGC CAC ACC GAG 1549
Tyr Val Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His Thr Glu
240 245 250

35 GAA CCG CCT GGA CTA AGT CTG GAT TTC CTG AAG TGC TGC AAG AGG AAT 1597
Glu Pro Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys Arg Asn
255 260 265 270

40 ACG GCC GAG GAA GAG AAC TCT GCA AAC CCT AAC CAA GAC CAG AAC GCA 1645
Thr Ala Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln Asn Ala
275 280 285

CGC CGA AGA AAG AAG AAG GAG AGA CGT CCT AGG GGC ACC ATG CAG GCT 1693
Arg Arg Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met Gln Ala
290 295 300

45 ATC AAC AAT GAA AGA AAA GCT TCG AAA GTC CTT GGG ATT GTT TTC TTT 1741
Ile Asn Asn Glu Arg Lys Ala Ser Lys Val Leu Gly Ile Val Phe Phe
305 310 315

50 GTG TTT CTG ATC ATG TGG TGC CCA TTT TTC ATT ACC AAT ATT CTG TCT 1789
Val Phe Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile Leu Ser
320 325 330

55 GTT CTT TGT GAG AAG TCC TGT AAC CAA AAG CTC ATG GAA AAG CTT CTG 1837
Val Leu Cys Glu Lys Ser Cys Asn Gln Lys Leu Met Glu Lys Leu Leu
335 340 345 350

AAT GTG TTT GTT TGG ATT GGC TAT GTT TGT TCA GGA ATC AAT CCT CTG 1885
Asn Val Phe Val Trp Ile Gly Tyr Val Cys Ser Gly Ile Asn Pro Leu
355 360 365

60 GTG TAT ACT CTG TTC AAC AAA ATT TAC CGA AGG GCA TTC TCC AAC TAT 1933
Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Asn Tyr
370 375 380

TTG CGT TGC AAT TAT AAG GTA GAG AAA AAG CCT CCT GTC AGG CAG ATT 1981
 Leu Arg Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg Gln Ile
 385 390 395
 5 CCA AGA GTT GCC GCC ACT GCT TTG TCT GGG AGG GAG CTT AAT GTT AAC 2029
 Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn
 400 405 410
 10 ATT TAT CGG CAT ACC AAT GAA CCG GTG ATC GAG AAA GCC AGT GAC AAT 2077
 Ile Tyr Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser Asp Asn
 415 420 425 430
 GAG CCC GGT ATA GAG ATG CAA GTT GAG AAT TTA GAG TTA CCA GTA AAT 2125
 Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn
 15 435 440 445
 CCC TCC AGT GTG GTT AGC GAA AGG ATT AGC AGT GTG TGA GAAAGAACAG CAC 2177
 Pro Ser Ser Val Val Ser Glu Arg Ile Ser Ser Val *
 450 455
 20 AGTCCTTTTCT ACGGTACAAG CTACATATGT AGGAAAATTT TCTTCTTTAA TTTTCTGTGT 2237
 GGTCTTAACT AATGTAAATA TTGCTGTCTG AAAAAGTGTT TTTACATATA GCTTTGCAAC 2297
 25 CTTGTACTTT ACAATCATGC CTACATTAGT GAGATTTAGG GTTCTATATT TACTGTTTAT 2357
 AATAGGTGGA GACTAACTTA TTTTGATTGT TTGATGAATA AAATGTTTAT TTTTGCTCTC 2417
 CCTCCCTTCT TTCCTTCCTT TTTTCCTTTC TTCCTTCCTT TCTCTCTTTC TTTTGTGCAT 2477
 30 ATGGCAACGT TCATGTTTCAT CTCAGGTGGC ATTTGCAGGT GACCAGAATG AGGCACATGA 2537
 CAGTGGTTAT ATTTCAACCA CACCTAAATT AACAAATTCA GTGGACATTT GTTCTGGGTT 2597
 35 AACAGTAAAT ATACACTTTA CATTCTTGCT CTGCTCATCT ACACATATAA ACACAGTAAG 2657
 ATAGGTTCTG CTTTCTGATA CATCTGTCAG TGAGTCAGAG GCAGAACCTA GTCTTGTTGT 2717
 TCATATAGGG GAATTC 2733
 40

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 459 amino acids
 (B) TYPE: amino acids
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (iii) HYPOTHETICAL: NO
 (iv) ANTISENSE: NO
 (v) FRAGMENT TYPE: internal
 (vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Val Asn Leu Arg Asn Ala Val His Ser Phe Leu Val His Leu Ile
 1 5 10 15
 Gly Leu Leu Val Trp Gln Ser Asp Ile Ser Val Ser Pro Val Ala Ala
 20 25 30
 Ile Val Thr Asp Ile Phe Asn Thr Ser Asp Gly Gly Arg Phe Lys Phe
 35 40 45
 Pro Asp Gly Val Gln Asn Trp Pro Ala Leu Ser Ile Val Ile Ile Ile
 50 55 60

5 Ile Met Thr Ile Gly Gly Asn Ile Leu Val Ile Met Ala Val Ser Met 80
 65 Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser Leu Ala 95
 10 Ile Ala Asp Met Leu Val Gly Leu Leu Val Met Pro Leu Ser Leu Leu 110
 115 Ala Ile Leu Tyr Asp Tyr Val Trp 105 Pro Leu Pro Arg Tyr 125
 130 Val Trp Ile Ser Leu Asp Val 135 Leu Phe Ser Thr Ala Ser Ile Met His 160
 145 Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala Ile Arg Asn Pro Ile 175
 15 Glu His Ser Arg Phe Asn Ser Arg Thr Lys Ala Ile Met Lys Ile Ala 190
 180 Ile Val Trp Ala Ile Ser Ile Gly Val Ser Val Pro Ile Pro Val Ile 205
 195 Gly Leu Arg Asp Glu Glu Lys Val 200 Phe Val Asn Asn Thr Thr Cys Val 220
 210 Leu Asn Asp Pro Asn Phe Val 215 Ile Ile Gly Ser Phe Val Ala Phe Phe 240
 225 Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu Thr Ile Tyr Val 255
 230 Leu Arg Arg Gln Ala Leu Met Leu Leu His 235 Gly His Thr Glu Glu Pro 270
 245 Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys Arg Asn Ala Arg Arg 300
 260 Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln Asn Ala Ile Asn 315
 275 Arg Lys Lys Lys Glu Arg Arg 280 Pro Arg Gly Thr Met Gln Ala Phe Val Phe 320
 290 Asn Glu Arg Lys Ala Ser Lys Val Leu Gly Ile Val Phe Phe Val Leu 335
 305 Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile Leu Ser Val Tyr 350
 35 Cys Glu Lys Ser Cys Asn Gln Lys Leu Met Glu Lys Leu Leu Asn Val 365
 340 Phe Val Trp Ile Gly Tyr Val Cys Ser Gly Ile Asn Pro Leu Val Tyr 380
 355 Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser Asn Tyr Leu Arg 395
 370 Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg Gln Ile Pro Arg 400
 385 Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn Val Asn Ile Tyr 415
 45 Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser Asp Asn Glu Pro 430
 420 Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro Val Asn Pro Ser 445
 435 Ser Val Val Ser Glu Arg Ile Ser Ser Val * 455
 50 450

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CACCTAATTG GCCTATTGGT T

5

(2) INFORMATION FOR SEQ ID NO:4:

21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

20

AAGGATTGCC AGGAGAGACA G

(2) INFORMATION FOR SEQ ID NO:5:

21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

35

TTGGCCTATT GGT TTGGGAA T

40

(2) INFORMATION FOR SEQ ID NO:6:

21

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTISENSE: NO

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

55

GTCTGGGAAT TTGAAGCGTC CAC

23

WE CLAIM:

1. Isolated DNA encoding the serotonin 5HT_{2C} receptor, wherein said DNA encodes a serine at amino acid position 23 of said receptor.
2. The isolated DNA of Claim 1 wherein said DNA is in a vector.
- 5 3. The isolated DNA of Claim 1 wherein said DNA has the nucleic acid sequence of SEQ ID NO:1.
4. An isolated protein having the amino acid sequence of a serotonin 5HT_{2C} receptor, wherein said protein has a serine residue at amino acid position 23.
5. The isolated protein of Claim 4 wherein said protein has the amino acid sequence of SEQ ID NO:2.
6. A method for detecting the presence of DNA encoding an allelic variant of the serotonin 5HT_{2C} receptor, comprising:
 - 10 isolating DNA encoding the serotonin 5HT_{2C} receptor;
 - amplifying a region of said DNA that encodes amino acid number 23 of the serotonin 5HT_{2C} receptor; and
 - determining whether said isolated DNA encodes a serine residue at amino acid number 23, wherein
15 a serine residue at amino acid position 23 indicates the presence of an allelic variant of the serotonin 5HT_{2C} receptor.
7. The method of Claim 6 wherein said amplifying step comprises polymerase chain reaction amplification.
8. The method of Claim 6 wherein said amplification uses DNA primers that insert a new restriction
20 site near the codon that encodes amino acid number 23.
9. The method of Claim 8 wherein said restriction site is cleaved by HinfI.
10. The method of Claim 8 wherein said DNA primers are SEQ ID NOS: 5 and 6.
11. The method of Claim 6 wherein said determining step further comprises restriction enzyme digestion followed by gel electrophoresis.
- 25 12. The method of Claim 6 wherein said determining step includes denaturation of said isolated DNA.
13. The method of Claim 11 wherein said restriction enzyme is Rsa I.
14. The method of Claim 11 wherein said gel electrophoresis is on a Mutation Detection Enhancement gel.
15. The method of Claim 6 wherein said determining step further comprises nucleic acid sequencing
30 of the amplified DNA.
16. An antibody specific for the serotonin 5HT_{2C} allelic variant receptor protein wherein said receptor protein has a serine at amino acid position 23.
17. The antibody of Claim 16 wherein said antibody is a monoclonal antibody.
18. A method for detecting the presence of an allelic variant of the serotonin 5HT_{2C} receptor,
35 comprising:
 - providing a biological sample containing the serotonin 5HT_{2C} receptor;

contacting said sample with an antibody which specifically binds to a serotonin 5HT_{2C} receptor with a serine residue located at amino acid position 23; and

detecting the binding of said antibody to said receptor, wherein detectable binding indicates the presence of an allelic form of the serotonin 5HT_{2C} receptor.

5

19. The method of Claim 18 wherein said cells are human.

20. The method of Claim 18 wherein said cells are brain cells.

21. The method of Claim 18 wherein said antibody is specific for the extracellular loop of the first transmembrane domain of the serotonin 5HT_{2C} receptor.

10

22. The method of Claim 21 wherein said antibody is a monoclonal antibody.

23. The method of Claim 18 wherein said antibody is labelled.

24. The method of Claim 23 wherein said label is radioactivity.

25. The method of Claim 23 wherein said label is a fluorescent molecule.

26. The method of Claim 18 wherein said antibody is monoclonal.

15

27. The method of Claim 18 wherein said detecting step further comprises fluorescence activated cell sorting.

28. A method for detecting the presence of an allelic variant of the serotonin 5HT_{2C} receptor, comprising:

20

providing a biological sample containing the serotonin 5HT_{2C} receptor;

contacting said sample with an antibody which specifically binds to a serotonin 5HT_{2C} receptor having a cysteine residue located at amino acid position 23, and wherein said antibody does not bind a 5HT_{2C} receptor having a serine residue at position 23; and

detecting the absence of binding of said antibody to said receptor, wherein lack of detectable binding indicates the presence of an allelic form of the serotonin 5HT_{2C} receptor.

1/4

FIG. 1a

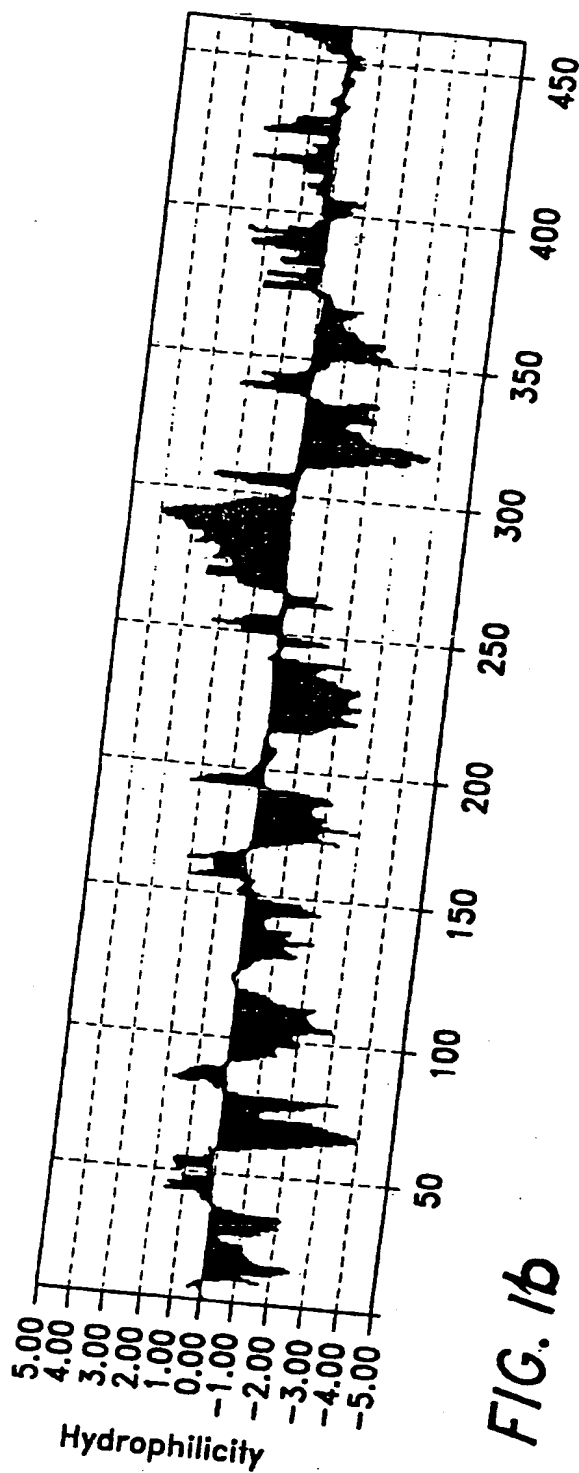


FIG. 1b

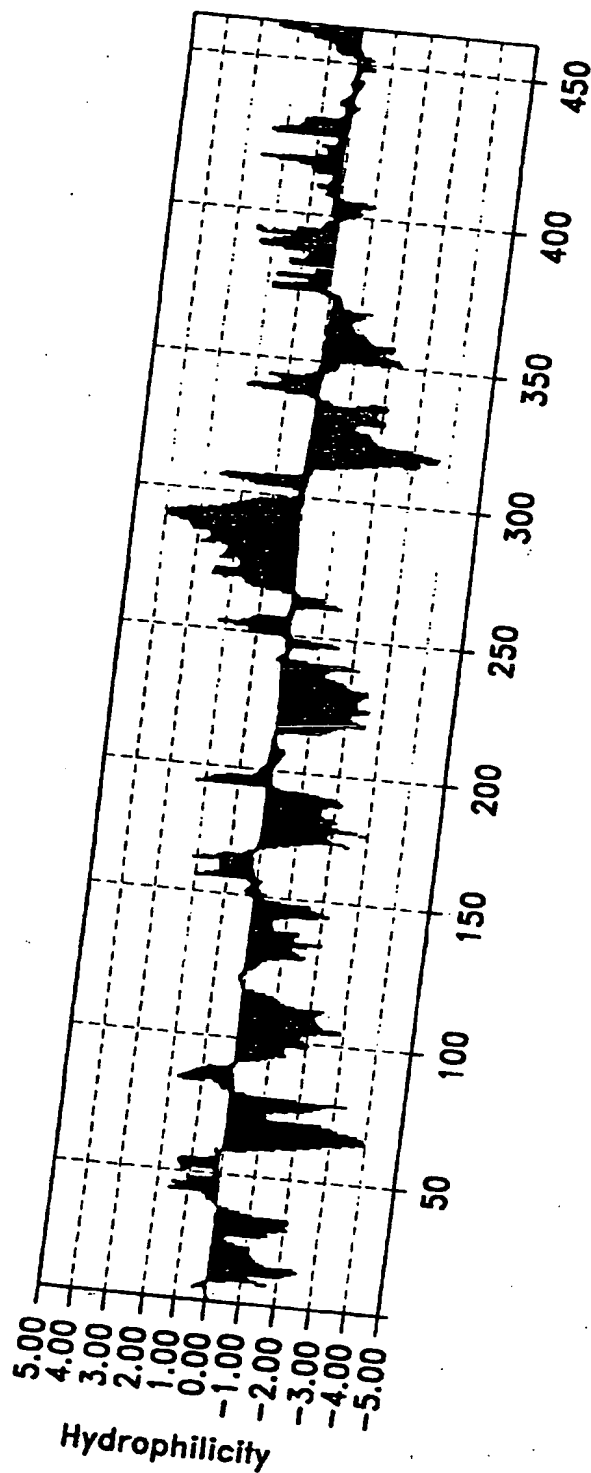
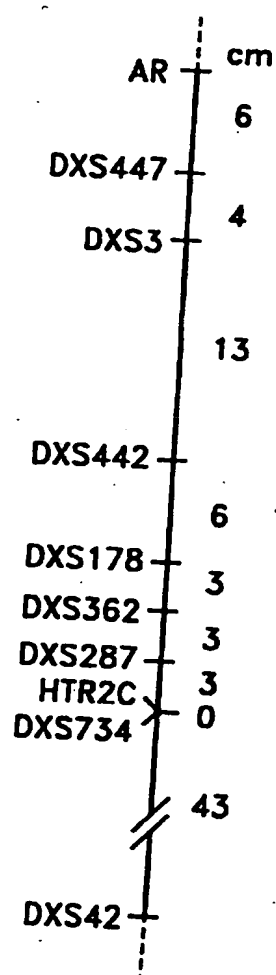


FIG. 2



3/4

FIG. 3a

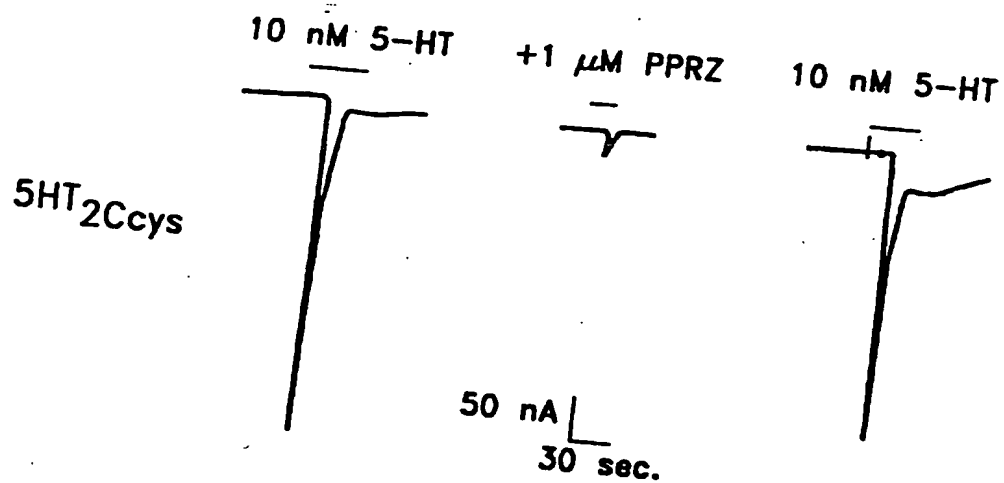


FIG. 3b

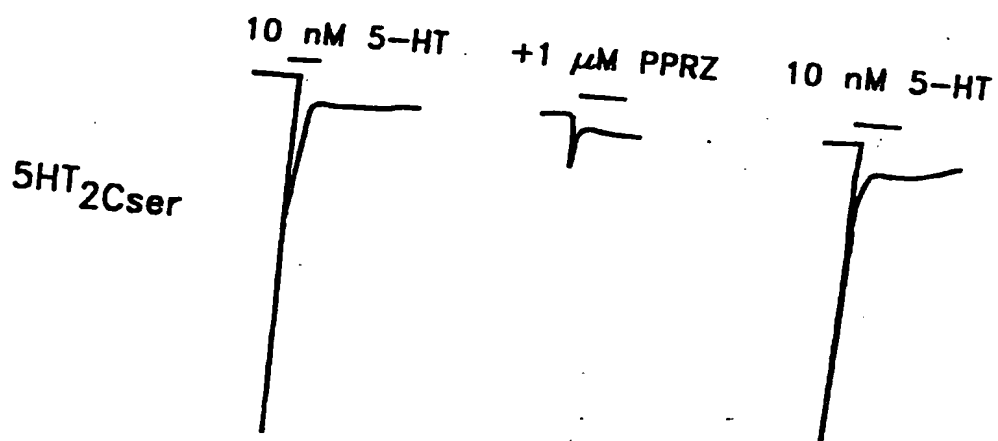


FIG. 4

